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Review article

# Diagnosis and treatment approaches to CMV infections in adult patients

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**Abstract**

*Background:* Cytomegalovirus (CMV) infections are very common in the general population. Clinical CMV disease, particularly CMV pneumonitis, greatly impacts the morbidity and mortality of immunosuppressed patients. *Objective:* To present an overview of the basic aspects of the biology, epidemiology, and clinical features of CMV in relation to the available diagnostic and therapeutic approaches in adult patients. *Methods:* Review of the medical literature on cytomegalovirus infection and disease in adult hosts, with a focus on approaches to diagnosis and treatment of CMV respiratory disease in immunosuppressed hosts. *Conclusions:* Cytomegalovirus infections are likely to remain a significant cause of morbidity and mortality among immunosuppressed patients. Important aspects of the biological events underlying the transition from infection to clinical disease remain unclear. Despite that, considerable progress has been made in the design of improved diagnostic techniques and the development of antiviral agents. Preventive and particularly preemptive therapeutic approaches demand further technical improvements in diagnostic testing. At present, the emphasis in the search for improved diagnostic testing rests on the development of quantitative methods for early detection of the increased viral replicative activity that presumably precedes the onset of CMV disease in infected individuals. © 2002 Elsevier Science B.V. All rights reserved.

*Keywords:* Cytomegalovirus; Pneumonia; Human immunodeficiency virus

## 1. Introduction and scope

Cytoemgalovirus (CMV) remains a major concern in the medical management of immunosuppressed individuals. It is estimated, for instance, that one third of transplant recipients experience CMV disease; up to half will have lung infections, resulting in a patient mortality rate as high as 50% (Goodrich et al., 1991; Schmidt et al., 1991). HIV-1 infected patients with $CD4^+$ cell counts below

*Abbreviations:* AIDS, acquired immunodeficiency syndrome; CMV, cytomegalovirus; CPE, cytopathogenic effect; HIV-1, human immunodeficiency virus 1; PCP, *Pneumocystis carinii* pneumonia; PCR, polymerase chain reaction; BMT, bone marrow transplant.

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100 μl remain at high risk for CMV disease despite the significant benefits of highly active antiretroviral therapy. Progress in the diagnosis and treatment of CMV infections has been achieved in recent years, although significant gaps remain in our understanding of the interactions between the virus and its host, the natural history of the infection, particularly the latent form, and the biological mechanism of its reactivation.

Clinically, the most controversial issue remains the differentiation between CMV infection and CMV disease. We will discuss aspects of CMV infections with a focus on adult respiratory diesase. Infections in children have been recently reviewed (Numazaki and Chiba, 1997).

## 2. Virology

CMV is a member of the Herpesviridae family. Its 230 kb double-stranded DNA genome, the largest of the herpes virsuses, is divided into two unequally sized unique and short regions, UL and US, respectively, flanked by repeat sequences. DNA homology of clinical strains is at least 80% (Huang et al., 1976). The significance of strain differences is not understood although reinfection of latently infected individuals by other cytomegalovirus (CMV) strains is known to occur. Strain variations can be identified by conventional methods such as neutralization kinetics and restriction endonuclease analysis, a particularly useful technique in epidemiologic studies.

Gene expression takes place in three overlapping phases, immediate-early, early and late, and produces more than 200 proteins. As with other herpes viruses, CMV immediate-early genes are thought to be stimulated by tegument proteins. Immediate-early transcripts precede a cascade of early proteins that result in genomic replication. The best characterized of these are DNA polymerase and thymidine kinase. Late gene products, primarily structural proteins, are less well understood.

CMV DNA is surrounded by three distinct layers: a matrix or tegument, a capsid, and an outer envelope. The icosahedral capsid, formed by capsomere subunits, encloses an amorphous tegument consisting of three predominant phosphoproteins: pp150, pp65 and pp71, derived from genes UL32, UL83 and UL82, respectively. Their function is not known; however, they are antigenic and early markers of viral infection, a feature that has proven useful in diagnosis, in particular with the pp65 antigenemia assay. The outer envelope derives from host cell nuclear and cytoplasmic membranes, and consists of three distinct families of viral glycoprotein complexes, gCI, gCII and gCIII. The host cell receptors which bind envelope glycoproteins have not been described; however, antibodies to glycoproteins neutralize infectivity, probably by blocking viral attachment.

## 3. CMV infection and disease

CMV infection is common in the general population with reported seroprevalences of 40–100% (Krech, 1973), in direct correlation with practices that favor exchanges of body fluids. Age-specific seroprevalence studies show a bimodal distribution with the first peak of seropositivity occurring in early childhood and a second one in young adulthood. The first peak results in part from vertical transmission (reviewed in Numazaki and Chiba, 1997). Frequent viral shedding in urine and respiratory secretions of infected children may account for horizontal transmission and the well-described daycare acquisition of CMV by adults and children. CMV seroconversion of young adults shows considerable geographic variation and is presumably determined largely by sexual transmission. The virus is highly prevalent among homosexual men and female prostitutes (Mintz et al., 1983; Dannenmaier et al., 1985).

CMV elicits both humoral and cellular immune responses. Viral phosphoproteins induce a more vigorous humoral immune response than glycoproteins. The biological role of the host's humoral immune response appears to be limited perhaps in relation to the intracellular nature of CMV infection, and viral immunoevasive mechanisms (Hengel et al., 1998). On the other hand, suppression of a host's cellular immune responses is the major underlying predisposing factor for CMV disease in adult populations that are targets for this virus:

patients with hematologic malignancies, therapeutically immunosuppressed transplant recipients, and individuals with late HIV-1 infection.

CMV presents as primary, latent, reactivated, and reinfection. As with other herpes viruses, latency follows all primary infections and is considered to be lifelong. Latent infection is the most prevalent CMV infection in the general population at any given time. Latently infected individuals may shed the virus in body fluids and transmit it to others; otherwise latency is of no consequence unless the host becomes immunosuppressed. The biologic mechanisms and host-agent interactions that allow latent infections remain largely unknown (Hengel et al., 1998). For the remainder of this article, the term 'CMV infection' will refer to the latent, asymptomatic form of the infection, and 'CMV disease' to the primary, reactivated, and reinfection types, where clinical symptoms and end-organ involvement are manifest.

Both viral and host characteristics are known to affect the occurrence and severity of CMV disease (whether primary, reactivation, or reinfection). Symptomatic CMV disease may occur at different times during the immunosuppressed state depending on a history of previous primary infection, the nature and severity of immune suppression, exposure, and, in the case of transplant patients, the occurrence of graft-versus host disease (GVHD) (Chou, 1987; Grundy et al., 1987; Ruutu et al., 1990; Ettinger et al., 1993). Recent reports suggest that viral properties, in particular immune-modulating factors directly affect the clinical presentation and course of CMV disease, as well as the occurrence and severity of other infections in immunosuppressed patients (Fries et al., 1994; Shepp et al., 1996; Eddleston et al., 1997).

### 3.1. Immunocompetent host

Respiratory infections and otherwise severe disease due to CMV are rare in immunocompetent hosts. Although primary infection is typically asymptomatic, it does occasionally present as a mononucleosis syndrome similar to Epstein–Barr virus (EBV) disease, with fever, malaise, myalgias, lymphocytosis, with or without pharyngitis or skin rashes (Klemola et al., 1970). In contrast to EBV disease, lymphadenopathy and splenomegaly are unusual, while mild hepatitis is frequently present. CMV hepatitis may also occur as an isolated primary infection. It is distinguished by a characteristic granulomatous reaction in liver tissue (Bonkowsky et al., 1984). Other clinical presentations with single or multiorgan involvement with or without mononucleosis can occur, including thrombocytopenia, hemolytic anemia, pneumonitis, polyradiculoneuritis, encephalitis and myocarditis (Ho, 1995).

### 3.2. Immunosuppressed host

CMV is an opportunistic agent for a variety of individuals with impaired cellular immunity, including cancer patients with solid or hematologic malignancies, individuals with late HIV-1 disease and therapeutically immunosuppressed patients, especially transplant recipients.

#### 3.2.1. Transplant patients

Recipients of both solid organ and marrow transplants are prone to develop a number of infectious and non-infectious complications. CMV disease typically occurs between the 30th and 100th post-transplant day. Past history of primary CMV infection, determined by pre-transplant serology on donors and recipients, greatly influences both the incidence and severity of CMV disease. CMV complications occur more frequently in patients known to be seropositive before transplantation, usually the result of reactivated latent infection but occasionally due to virus from the transplanted organ, or transfused blood or blood products. Disease in seronegative patients due to primary infection, while less frequent, is generally considered to be more severe (Ettinger et al., 1993).

CMV disease in the post-transplant period also varies according to the type of transplant, the nature and duration of immune suppression and the presence of GVHD. It tends to be less frequent but more severe in bone marrow transplant (BMT) patients than in recipients of solid-organ transplants (SOT). Cytotoxic immunosuppressants and depressors of T-cell function increase the risk of

disease to varying degrees; e.g. methotrexate, antilymphocyte globulin, OKT3 antisera, and total body irradiation are important risk factors, while corticosteroids or neutropenia alone are not. Tacrolimus may increase the risk of CMV disease (Maltezou et al., 1999), to a degree similar to that of cyclosporine (Hadley et al., 1995). Although mycophenolate mofetil seems not to increase the incidence of CMV infections (ter Meulen et al., 2000; Giral et al., 2001), it may increase the risk of disease in those infected ter Meulen et al., 2000. CMV disease is more likely when GVHD occurs but the converse is also true (Grundy et al., 1987; Sherlock et al., 1991). The association of CMV gB genotype with death due to myelosuppression was noted in one study of 281 CMV isolates from BMT recipients. Data from the same study also suggested that gB types 3 and 4 may be associated with a reduced risk of grades II–IV GVHD (Torok-Storb et al., 1997).

There is a wide range of clinical manifestations and organ involvement in post-transplantation CMV disease. The virus often targets the transplanted organ; hepatitis occurs in 40% of liver transplant recipients, and pneumonitis is more frequent in heart and heart–lung recipients (Drummer et al., 1985; Bronsther et al., 1988). Other targets include the gastrointestinal tract, and the peripheral and central nervous systems. CMV retinitis, although common in late HIV-1 disease, is unusual in transplant patients. A CMV syndrome, characterized by non-specific symptoms such as fever, leukopenia, thrombocytopenia, and transaminase elevations occurs commonly and may be difficult to distinguish from graft rejection.

CMV respiratory infection occurs in 17–38% of solid organ recipients (Bowden, 1993). While fewer marrow recipients experience CMV pneumonitis (7–20%), it is clearly more severe in this group of patients with mortality rates from 50 to 80% (Meyers et al., 1975). Presentation may be overt or insidious, but typically includes fever, dyspnea, hypoxia, and cough. Chest radiographs may demonstrate diffuse interstitial infiltrates, but these may not be found in half of all patients with documented CMV pulmonary infection (Ettinger et al., 1993). There are no specific clinical signs associated with CMV pneumonitis; supportive laboratory data are required for diagnosis.

### 3.2.2. *HIV-1 infected patients*

The success of potent antiretroviral therapy has resulted in a declining incidence of opportunistic infections in HIV-1 infected patients, but patients with $CD4^+$ counts less than 100 per μl remain at risk for CMV disease. CMV infection is highly prevalent in HIV-1 patients and has been reported as an independent predictor of mortality in AIDS (Gallant et al., 1992; Chevret et al., 1999). Retinitis is its most common manifestation accounting for 75–85% of all CMV disease in this group. Gastrointestinal disease is also common. Other manifestations include encephalitis and polyneuritis. Adrenal infection is a common postmortem diagnosis. Despite their importance, the natural history of these disorders is largely unknown.

Although CMV is frequently recovered from respiratory specimens of AIDS patients, its role as a respiratory pathogen and its influence on AIDS morbidity and mortality remain controversial (Murray et al., 1984; Pass et al., 1984; Wallace, 1989; Schooley, 1990). CMV is identified in about 50% of HIV-1-infected patients with pneumonia (Broaddus et al., 1985; de la Hoz et al., 1996) and autopsy series report findings of CMV pneumonitis in close to 60% of all the cases studied, but its true contribution to morbidity is unclear (Nash and Fligiel, 1984; Hui et al., 1984; Wallace and Hannah, 1987). In contrast to transplant patients, CMV pneumonitis is infrequently diagnosed premortem in HIV-1 patients, perhaps due to the relative infrequency of chest radiographic abnormalities (Ettinger et al., 1993).

CMV is a common co-pathogen in *Pneumocystis carinii* pneumonia (PCP). Reports of increased mortality in patients with both PCP and CMV have been reported by some but certainly not all investigators (Pass et al., 1984; Stover et al., 1985; Millar et al., 1990; Miles et al., 1990; Jacobson et al., 1991; Bozzette et al., 1992; de la Hoz et al., 1996). In the setting of HIV-1 infection, CMV pneumonitis results much more frequently from reactivation of latent virus than from a primary infection (Alford and Britt, 1985), and may occur as a pre-terminal event in the course of PCP. This

pre-terminal co-infection is thought by some investigators to contribute to the mortality from PCP (de la Hoz et al., 1996). At present, it is perhaps fair to say that although CMV occasionally causes pneumonitis in HIV-1-infected patients, it is more often associated with a chronic, subclinical respiratory infection, with an uncertain influence on the morbidity and mortality of HIV-1 infection or PCP.

## 4. Diagnosis of CMV infection and disease

With the notable exception of retinitis, CMV disease cannot be diagnosed on clinical grounds alone. While viral isolation from patient samples establishes the diagnosis of CMV infection, the diagnosis of CMV disease is more complex, particularly in the immunosuppressed host. Classically it has required evidence of the virus in end-organ tissue, cerebrospinal fluid, or blood, using histopathology, cytology or culture. Immunocompromised patients present a challenge because reactivation of a latent virus is common, and relying on tissue diagnoses places patients at risk from invasive procedures and delayed treatment. CMV detection in urine and respiratory secretions alone is often not sufficiently diagnostic of CMV disease (Ruutu et al., 1990; Drew, 1992; de la Hoz et al., 1998), although it is considered highly suggestive in seropositive donor/seronegative recipient organ transplantation cases (Ettinger et al., 1993).

### *4.1. Diagnostic tests*

The most interesting driving force behind technological advances in CMV diagnostic methods, has been the search for markers of active CMV disease. These markers may be qualitative, e.g. detection of CMV genome, activation of a particular viral gene (Boriskin et al., 2002), appearance of its product, etc. More efforts seem to have been devoted, however, to the finding of quantitative markers, particularly an increase in circulating viral load, which presumably characterizes the transition to an active CMV disease.

Advances in monoclonal antibody technology and the proliferation of molecular methods in the last two decades have produced diagnostic tests which have decreased time and improve predictive values for a number of viral diagnoses including CMV. Since the newer test methods allow laboratories to quantitate viruses, it may be possible for clinicians to distinguish disease from infection, monitor therapy and establish therapeutic endpoints. Rapid methods may also permit the selective use of antivirals in transplant recipients and HIV-1 patients.

Diagnostic tests in current use for CMV diagnosis include the following.

#### *4.1.1. Viral culture*

CMV has been traditionally isolated in fibroblast tissue culture where it produces a distinctive cytopathogenic effect (CPE) that is easily confirmed by fluorescent antibody stains. It can be recovered from a variety of patient specimens-urine, tissues, respiratory swabs and washes, body fluids and blood. Conventional viral culture has been the gold standard for diagnosing CMV despite shortcomings such as poor predictive value, lack of quantitation, and 1–3 weeks turnaround time. Falsely negative cultures may occur if the interval between specimen collection and cell culture inoculation is prolonged. Cell cultures have a lower sensitivity when compared with polymerase chain reaction (PCR) and nucleic acid probe methods (Sandin et al., 1991), and the latter may thus become the 'gold standard.' An important and often overlooked benefit of viral culture compared with molecular methods is the ability to recover other viruses from patient specimens.

Rapid viral culture (shell vial method) is a modification of conventional culture that has reduced report time to 48 h from 7 to 14 days. In this method, specimens are centrifuged onto fibroblast monolayers, incubated briefly, then stained before the appearance of CPE with a monoclonal antibody to early antigens (Hudson et al., 1976; Gleaves et al., 1985). Sensitivity is reported to be 68–100% compared with conventional culture (Hudson et al., 1976; Gleaves et al., 1985; Gerna et al., 1990; Rabella and Drew, 1990; Mazzulli et al., 1993; Lipson et al., 1993). Most

laboratories continue to do parallel conventional cultures. Quantitative shell vial culture methods are available but are not widely used (Gerna et al., 1991).

*4.1.2. Nucleic acid detection*

Nucleic acid probes and amplification methods such as PCR are increasingly important for viral diagnoses. Probes may be used to detect CMV genes directly from clinical samples or after amplification by culture or PCR. Traditional nucleic acid hybridization assays (in-situ or on filters) have not realized their potential in CMV diagnosis, except, perhaps, on histopathological samples (Myerson et al., 1984; Keh and Gerber, 1988; Joundi et al., 1991). Initially optimistic reports with in-situ hybridization on cells from BAL fluid were not confirmed in subsequent studies on transplant recipients and HIV-1 patients (Hillborne et al., 1987; Agut et al., 1988; Gleaves et al., 1989; de la Hoz et al., 1998).

Direct hybridization methods have also been used to detect viral genome after nucleic acid extraction. Studies on two commercially available methods, the CMV hybrid capture assay (Digene, Beltsville, MD), and the branched-DNA probe assay (Chiron, Emeryville, CA), have been reported. In the hybrid capture (HC) assay, unlabeled CMV probes hybridize with complementary viral DNA. Subsequently, hybrids are immobilized on a solid phase, then measured by conjugated anti-hybrid antibody. The branched DNA assay makes use of an artificial molecule to amplify the signal of the bound probe. These tests have been applied with good results to blood and spinal fluid specimens (Mazzulli et al., 1996; Flood et al., 1997). Utility for diagnosing pneumonia remains to be established.

PCR has also been proposed as a method of diagnosing disseminated and end-organ CMV disease. Numerous studies have been published comparing the performance of qualitative in-house PCR methods with conventional culture and antigenemia. Results have been inconsistent. Despite the high sensitivity and negative predictive values, several studies suggest clinical utility is limited by low positive predictive values (Gerna et al., 1991; Delgado et al., 1992; Storch et al., 1994). Proposed remedies include improved quantitation, amplification of CMV messenger RNA, or late transcripts (Gozlan et al., 1993; Meyer-Konig et al., 1995; Boriskin et al., 2002). Ultimately, the improved availability of standardized, cost-effective, quantitative PCR kits will determine the utility of this method.

Turnaround time for both PCR and nucleic acid probe assays is 6–48 h; however, current kit configurations would require most laboratories to delay testing while a sufficiently large batch of specimens is accumulated to allow cost-effective testing.

*4.1.3. Antigen detection*

CMV antigenemia was developed in 1988 by Van der Bij to improve detection of CMV viremia in transplant recipients (van der Bij et al., 1988). The test detects the CMV pp65 antigen in circulating leukocytes of fresh anticoagulated blood or spinal fluid. Leukocytes are washed, counted, and $1.5 \times 10^5$ are spotted in duplicate onto a multi-welled glass slide. After fixation and staining with anti-pp65 monoclonal fluorescein or peroxidase conjugate, the number of labeled cells is counted. Evaluations of this assay have shown better or equivalent sensitivity and predictive values compared with culture (Mazzulli et al., 1993; Landry and Ferguson, 1993).

Some, but not all, investigators found that the antigenemia test was able to detect CMV disease before the onset of symptoms and to revert to negative with successful therapy (van der Bij et al., 1988; van den Berg et al., 1990; Grossi et al., 1995; Gratacap-Cavallier et al., 1995; Meyer-Konig et al., 1995). A significant limitation of the test is a requirement for prompt, specimen processing: leukocytes must be separated within 6–8 h of collection. It is also labor intensive, and requires skilled personnel to read and interpret the test. Inexpensive kits are commercially available, and results are available within 8–24 h.

Although direct antigen detection has been successful with blood and cerebrospinal fluid samples, success has been mixed when applied to other specimens. Antigen detection in urine is hampered by poor sensitivity and non-specific fluorescence (McKeating et al., 1985; Lucas et

al., 1989). Direct antigen detection on BAL samples was initially reported to have 100% sensitivity in a small number of BMT patients, but other studies failed to corroborate that finding (Emanuel et al., 1986; Martin and Smith, 1986; Gleaves et al., 1989; Weiss et al., 1991). Immunohistochemistry remains useful in open lung material, which is presently rarely obtained in immunosuppressed patients (Myerson et al., 1984).

#### 4.1.4. *Histology/cytology*

Cytomegalic intranuclear (owl's eye) inclusions in tissue are pathognomonic for CMV infection; intracytoplasmic inclusions are also seen in CMV infected cells. Lung, colon, esophageal, and liver have been common biopsy sites. Particularly in the case of lung biopsies, however, while histopathologic diagnosis is specific and confirms end-organ disease, false negatives due to sampling error are common. The difficulty in differentiating CMV from other viral and non-viral inclusions, and the requirement for invasive sampling techniques further limits the usefulness of this diagnostic modality. Alveolar cells are the primary sites of pulmonary CMV infection. BAL allows sampling from multiple lung segments, and the cells obtained can be washed, concentrated, and stained for inclusions. At least one report demonstrated the superiority of cytologic examination of BAL specimens in comparison to lung biopsy (Cordonnier et al., 1987).

#### 4.1.5. *Serology*

Serology, while useful for epidemiologic studies, has limited value in the diagnosis of CMV disease with the notable exception of primary infection when seroconversion establishes primary disease. Serologic diagnosis is best achieved with sensitive methods such as enzyme immunoassay (EIA), fluorescent antibody (FA), and anticomplement immunofluorescence (ACIF). Insensitive serologic methods such as complement fixation (CF) are unreliable (Waner et al., 1973). Although IgM assays are used in the diagnosis of primary disease for many infections, they must be interpreted with caution in the case of CMV. CMV IgM may be associated with reactivation of latent virus, particularly in HIV-1-infected patients (Mintz et al., 1983). Many immunocompromised patients do not mount an IgM response either due to underlying illness or antiviral agents. On the other hand, some patients will have reactive CMV IgM up to a year after acquiring CMV infection (Harzic et al., 1987; Lazzarotto et al., 1992).

Establishing CMV serologic status of pre-transplant patients and donors is an essential part of an initial assessment. Since seronegative patients who acquire primary infection in the course of transplantation and are at high risk of severe disease, they should be identified early and monitored closely for seroconversion or infection during the post-transplant period. Ideally, baseline sera should be stored through the early post-transplant period in the event it is required for CMV or other comparative titred assays. Primary infection may be impossible to diagnose serologically if sera are drawn after transfusions of blood or blood products in the post-transplant period.

### 4.2. *Antiviral prophylaxis and pre-emptive therapy*

Until recently, the lack of reliable tests to predict or accurately diagnose early CMV disease, led transplant centers to develop morbidity and mortality risk reduction strategies which relied on donor selection, use of CMV negative or irradiated blood products, management of GVHD, antibiotic and antiviral prophylaxis. While many programs vary in approach to patient selection, timing and duration of CMV prophylactic regimens, most utilize antivirals, usually acyclovir or ganciclovir and more recently foscarnet (Bacigalupo et al., 1994; Ippoliti et al., 1997), with or without immune globulin. Viral prophylaxis is typically administered 1–3 months in the immediate post-transplant period (Merigan et al., 1992; Goodrich et al., 1993; Winston et al., 1993; Martin et al., 1994). While no single strategy is efficacious for all types of transplants the success of ganciclovir prophylaxis is well documented. The disadvantage is that this approach provides treatment to more patients than those who would truly benefit from it.

Early prophylactic treatment of CMV infection has been proposed in AIDS (Mazzulli et al., 1993; Lipson et al., 1993; Landry and Ferguson, 1993),

In the setting of HIV infection, this approach would be more accurately described as pre-emptive (as defined below). The cost of this approach has prevented its implementation. A more pressing unresolved question is how to appropriately select patients for treatment, e.g. all symptomatic AIDS patients, those with markers of CMV disease, or those with clinical evidence of end-organ damage (Spector et al., 1996; Whitley et al., 1998). Prolonged (sometimes indefinite) courses of CMV antivirals are routine for treatment of active disease in this population, given the tendency to relapse. The duration of anti-CMV treatment is influenced by factors such as HIV disease activity and treatment toxicity. The use of highly active antiretroviral treatments improves the response to CMV infection and disease in AIDS patients.

Despite the success of these regimens, drug toxicity and antiviral resistance are significant limitations. Up to 40% of patients on ganciclovir will experience marrow suppression, often as early as the second week of therapy. Up to one third of the patients treated with foscarnet will develop side effects, notably dose-limiting nephropathy, metabolic abnormalities, and central nervous system adverse effects. Hydration may minimize nephrotoxicity. More concerning are reports of treatment failure due to resistant virus emerging during prolonged therapy (Jabs et al., 1998; Drew et al., 1999; Limaye et al., 2000).

Problems with drug toxicity and resistance, added to considerations of costs, have provided incentives for the development of alternative antiviral strategies, especially preemptive treatment. This involves initiating therapy only when there is supporting laboratory evidence of disease, ideally before clinically evident or severe end-organ damage. Results have been encouraging, but not uniformly so (Goodrich et al., 1991; Schmidt et al., 1991; Boeckh et al., 1996). Clearly, the success of this strategy will depend on optimizing diagnostic protocols that are supported by laboratory data. Virology laboratories are faced with selecting cost-effective, accurate, rapid tests able to detect early disease or increased viral replicative activity to allow effective management of patients at risk. Considerable controversy remains about the laboratory test(s) and specimens to be employed for this purpose, and about the timing of these tests in relation to immunosuppression or emergence of symptoms.

### 4.3. Laboratory strategies

Despite significant progress during the last decade, our understanding of the natural history of CMV-host interactions remains incomplete, and those gaps in our knowledge underlie the many remaining controversies about the diagnosis and treatment of CMV disease. At present, the innovative developments in CMV detection techniques hold more promise as insightful research tools than as clinically reliable diagnostic methods. Indeed, the limitations in assessing their clinical reliability partly relate to lack of a consensus on definitions of CMV disease.

In transplant recipients, the diagnostic approach to CMV pneumonia may be modified by a stratification of risk of disease and/or mortality, according to the pre-transplantation serological status of the donor and recipient. A less stringent case definition of disease and a lower threshold for intervention, including perhaps pre-emptive treatment, would apply to the seropositive donor/seronegative recipient patient. Post-transplant follow-up surveillance (by antigenemia, PCR, or viremia) contributes to identification of higher risk patients. Although chest radiographic abnormalities increase the likelihood of CMV pneumonia, the clinician needs to consider that X-ray findings may be subtle or even absent. On the other hand, the presence of CMV in bronchial lavage or transbronchial biopsy specimens should be required for lower risk patients, bearing in mind that positive findings may lack clinical significance, at least therapeutically, in asymptomatic patients.

In HIV-1-infected patients, no definitive approach to the diagnosis of CMV pneumonia has emerged. Presently the following criteria may be used (Drew, 1992): (1) exclusion of potential alternative pathogens and/or diagnoses; (2) detection of CMV in pulmonary secretions or tissue; (3) presence of characteristic CMV inclusions in bronchoalveolar lavage macrophages or lung tissue biopsies.

For both major groups of immunosuppressed patients, research is presently aimed at finding better markers of clinically significant disease. Since the enthusiasm about pre-emptive treatment strategies, there is presently more emphasis on quantitative markers. Although many of the newer assays are quantifiable, thresholds to establish presence of disease have not been reliably established. Research into qualitative markers of disease is much less developed.

The development of effective and accurate tests to diagnose and monitor patients at risk for CMV disease is expected to continue in parallel with the development of effective CMV antivirals, a pattern similar to what has already evolved for HIV-1, and is evolving for hepatitis B and C. In the future, however, laboratories can anticipate more scrutiny as health care providers struggle to reconcile demands of a growing number of ill patients and finite health care resources.

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